IN THE SPECIFICATION

Please amend the specification as follows:

Please replace the following paragraphs:

Page 3, first paragraph after the heading, "BACKGROUND:"

Extensive surveys of the fatty acid composition of seed oils from different species of higher plants have resulted in the identification of at least 33 structurally distinct monohydroxylated plant fatty acids, and 12 different polyhydroxylated fatty acids that are accumulated by one or more plant species (reviewed by van de Loo *et al.* 1993). Ricinoleic acid, the principal constituent of the seed oil from the castor plant *Ricinus communis (L.)*. is of commercial importance. We have previously described the cloning of a gene from this species that encodes a fatty acid hydroxylase, and the use of this gene to produce ricinoleic acid in the transgenic plants of other species (see U.S. patent application Serial No. 08/320,982, filed October 11, 1994**, now U.S. Patent No. 5,801,026**). The scientific evidence supporting the claims in that patent application were subsequently published (van de Loo *et al.*, 1995).

Page 10, third paragraph after the heading, "Conceptual basis of the invention:"

In U.S. patent application No. 08/320,982**, now U.S. Patent No. 5,801,026**, we described the use of a cDNA clone from castor for the production of ricinoleic acid in transgenic plants. As noted above, biochemical studies by others had suggested that the castor hydroxylase may not have strict specificity for oleic acid but would also catalyze hydroxylation of other fatty acids such as icosenoic acid (20:1 $^{cis\Delta 11}$) (Howling *et al.*, 1972). Based on these studies, our previous application No. 08/320,982**, now U.S. Patent No. 5,801,026** noted in Example 2 that the expression of the castor hydroxylase in transgenic plants of species such as *Brassica napus* and *Arabidopsis thaliana* that accumulate fatty acids such as icosenoic acid ((20:1 $^{cis\Delta 11}$) and erucic acid (13-docosenoic acid; 22:1 $^{cis\Delta 13}$) would be expected to accumulate some of the hydroxylated derivatives of these fatty acids due to the activity of the hydroxylase on these fatty acids. We have

now obtained additional direct evidence for such a claim based on the production ricinoleic, lesquerolic, densipolic and auricolic fatty acids in transgenic Arabidopsis plants and have included such evidence herein as Example 1.

Page 12-13, first paragraph:

In view of the high degree of sequence similarity between $\Delta 12$ fatty acid desaturases and the castor hydroxylase (van de Loo *et al.*, 1995), the validity of claims (*e.g.*, PCT WO 94/11516) for the use of desaturase or hydroxylase genes or sequences derived therefrom for the identification of genes of identical function from other species must be viewed with skepticism. In this application, we teach a method by which hydroxylase genes can be distinguished from desaturases and describe methods by which $\Delta 12$ desaturases can be converted to hydroxylases by the modification of the gene encoding the desaturases. A mechanistic basis for the similar reaction mechanisms of desaturases and hydroxylases was presented in the earlier patent application (No. 08/320,982**, now U.S. Patent No. 5,801,026**). Briefly, the available evidence suggests that fatty acid desaturases have a similar reaction mechanism to the bacterial enzyme methane monooxygenase which catalyses a reaction involving oxygen-atom transfer ($CH_4 \rightarrow CH_3OH$) (van de Loo *et al.*, 1993). The cofactor in the hydroxylase component of methane monoxygenase is termed a μ-oxo bridged diron cluster (FeOFe). The two iron atoms of the FeOFe cluster are liganded by protein-derived nitrogen or oxygen atoms, and are tightly redox-coupled by the covalently-bridging oxygen atom. The FeOFe cluster accepts two electrons, reducing it to the diferrous state, before oxygen binding. Upon oxygen binding, it is likely that heterolytic cleavage also occurs, leading to a high valent oxoiron reactive species that is stabilized by resonance rearrangements possible within the tightly coupled FeOFe cluster. The stabilized high-valent oxoiron state of methane monooxygenase is capable of proton extraction from methane, followed by oxygen transfer, giving methanol. The FeOFe cofactor has been shown to be directly relevant to plant fatty acid modifications by the demonstration that castor stearoyl-ACP desaturase contains this type of cofactor (Fox *et al.*, 1993).

Page 14-15, third paragraph:

Taking these three arguments together, it was hypothesized that kappa hydroxylase of castor endosperm is homologous to the microsomal oleate Δ12 desaturase found in all plants. The evidence supporting this hypothesis was disclosed in the previous patent application (No. 08/320,982**, now U.S. Patent No. 5,801,026**). A number of genes encoding microsomal Δ12 desaturases from various species have recently been cloned (Okuley *et al.*, 1994) and substantial information about the structure f these enzymes is now known (Shanklin *et al.*, 1994). Hence, in the following invention we teach how to use structural information about fatty acyl desaturases to isolate kappa hydroxylase genes of this invention. This example teaches the method by which any carbon-monoxide insensitive plant fatty acyl hydroxylase gene can be identified by one skilled in the art.

Page 49, first and third paragraphs:

The kappa hydroxylase encoded by the previously described fah12 gene from Castro (U.S. Patent application 08/320,982**, now U.S. Patent No. 5,801,026**) was used to produce ricinoleic acid, lesquerolic acid, densipolic acid and auricolic acid in transgenic Arabidopsis plants. This example specifically discloses the method taught in Example 2 of U.S. Patent application 08/320,982**, now U.S. Patent No. 5,801,026**.

Arabidopsis plants were transformed, by *Agrobacterium*-mediated transformation, with the kappa hydroxylase encoded by the Castor fah12 gene on binary Ti plasmid pB6. This plasmid was previously used to transform *Nicotiana tabacum* for the production of ricinoleic acid (U.S. Patent application 08/320,982**, now U.S. Patent No. 5,801,026**).

Pages 58-59, first paragraph:

The presence of lesquerolic acid in the transgenic plants was anticipated in the previous patent application (No. 08/320,982, **now U.S. Patent No. 5,801,026**) based on the biochemical evidence suggesting broad substrate specificity of the kappa hydroxylase. By contrast, the accumulation of densipolic and auricolic acids was less predictable. Since Arabidopsis does not normally contain significant quantities of the non-hydroxylated precursors of these fatty acids which could serve as substrates for the hydroxylase, it appears that one or more of the three n-3 fatty acid desaturases known in Arabidopsis (*e.g.*, fad3, fad7, fad8; reviewed in Gibson *et al.*, 1995) are capable of desaturating the hydroxylated compounds at the n-3 position. That is, densipolic acid is produced by the action of an n-3 desaturase on ricinoleic acid. Auricolic acid is produced by the action of an n-3 desaturase on lesquerolic acid. Because it is located in the endoplasmic reticulum, the fad3 desaturase is almost certainly responsible. This can be tested in the future by producing fah12- containing transgenic plants of the fad3-deficient mutant of Arabidopsis (similar experiments can be done with fad7 and fad8). It is also formally possible that the enzymes that normally elongate 18:1 $^{cis\Delta 9}$ to 20:1 $^{cis\Delta 11}$ may elongate 12OH-18:1 $^{cis\Delta 9}$ to 14OH-20:1 $^{cis\Delta 11}$, and 12OH-18:1 $^{cis\Delta 9, 15}$ to 14OH-20:1 $^{cis\Delta 11, 17}$.

Pages 60-62, first paragraph:

Although Arabidopsis is not an economically important plant species, it is widely accepted by plant biologists as a model for higher plants. Therefore, the inclusion of this example is intended to demonstrate the general utility of the invention described here and in the previous application (No. 08/320,982, **now U.S. Patent No. 5,801,026**) to the modification of oil composition in higher plants. One advantage of studying the expression of this novel gene in Arabidopsis is the existence in this system of a large body of knowledge on lipid metabolism, as well as the availability of a collection of mutants which can be used to provide useful information on the biochemistry of fatty acid hydroxylation in plant species. Another advantage is the ease of transposing any of the information obtained on metabolism of ricinoleate in Arabidopsis to closely related

species such as the crop plants *Brassica napus, Brassica juncea* or *Crambe abyssinica* in order to mass produce ricinoleate, lesqueroleate or other hydroxylated fatty acids for industrial use. The kappa hydroxylase is useful for the production of ricinoleate or lesqueroleate in any plant species that accumulates significant levels of the precursors, oleic acid and icosenoic acid. Of particular interest are genetically modified varieties that accumulate high levels of oleic acid. Such varieties are currently available for sunflower and Canola. Production of lesquerolic acid and related hydroxy fatty acids can be achieved in species that accumulate high levels of icosenoic acid or other long chain monoenoic acids. Such plants may in the future be produced by genetic engineering of plants that do not normally make such precursors. Thus, we envision that the use of the kappa hydroxylase is of general utility.

Pages 85-86:

(*e.g.*, cytochrome b5) or for the regulation of desaturase activity. We envision that the interaction between the hydroxylase and this other protein suppresses the activity of the desaturase. For instance, the quaternary structure of the membrane-bound desaturases has not been established. It is possible that these enzymes are active as dimers or as multimeric complexes containing more than two subunits. Thus, if dimers or multimers formed between the desaturase and the hydroxylase, the presence of the hydroxylase in the complex may disrupt the activity of the desaturase. this general hyprothesis will be tested directly by the production of transgenic plants in which the hydroxylase enzyme has been rendered inactive by the elimination of one or more of the histidine residues that have been proposed to bind iron molecules required for catalysis. Several of these histidine residues have been shown to be essential for catalysis by site directed mutagenesis (Shanklin *et al.*, 1994). codons encoding histidine residues in the castor hydroxylase gene described in U.S. patent application 08/320,982 will be changed to alanine residues as described by Shanklin *et al.*, (1994). The modified genes will be introduced into transgenic plants of Arabidopsis and possibly other species such as tobacco by the methods described in Example 1 of this application or in Example 1 of the

original version of this application (U.S. application 08/320,982**, now U.S. Patent No. 5,801,026**).